Proceedings of the American Academy of Arts and Sciences.

Vol. 60. No. 9.—November, 1925.

ON THE SYMBIOSIS OF CERTAIN BERMUDA COELENTERATES AND ZOOXANTHELLAE.

By H. Boschma.

CONTRIBUTIONS FROM THE BERMUDA BIOLOGICAL STATION FOR RESEARCH. NO. 150.

By H. Boschma.

ON THE SYMBIOSIS OF CERTAIN BERMUDA COELENTERATES AND ZOOXANTHELLAE.

Received November 19, 1924. Presented by E. L. Mark November 12, 1924.

The association of coelenterates with unicellular algae (zooxanthellae or zoochlorellae) is usually defined as a kind of symbiosis. There are, however, only very few data on the advantages which each of the two organisms obtains from this association. In a previous paper (Boschma, 1924) I have published the results of a study of the contents of the gastric cavity in a large number of East Indian coral-polyps. In these contents I often found partly digested foreign organisms, or parts of them, and besides these invariably zooxanthellae in different stages of disintegration. Thus I have confirmed the results of Gardiner (1903), who found that most of the reef-corals derive a considerable part of their food from their symbiotic algae, and even that the polyps of many species feed exclusively on their zooxanthellae.[1]

To give definite proof that the zooxanthellae are digested by the polyps of reef-corals and by other coelenterates it was important that the process of digestion should be studied in living polyps more accurately than has been done hitherto. I was therefore glad of the opportunity to work for some time at the Bermuda Biological Station for Research during the latter half of September, 1924. I am greatly indebted to the Director of this Station, Dr. E. L. Mark, who allowed me to use the laboratory after it was already closed, and who kindly revised the manuscript of this paper. I also wish to thank Dr. Roy W. Miner, who loaned me a microscope of American manufacture, which could be brought back to the United States without paying importation duties.

As I could spend only a short time (ten days) in the Bermudas, and as the weather during this period was too rough to permit the collecting of corals at the outer reefs, I confined my experiments to coelenterates which were easily to be collected in the neighborhood of Agar's Island. The four which I studied were: *Isophyllia dipsacea* (Dana), *Siderastraea radians* (Pallas), *Zoanthus sociatus* (Ellis), and *Condylactis passiflora* Duch. et Mich.

The polyps were kept alive in large glass jars containing sea-water that was renewed every day. This, however, did not seem to be quite sufficient to keep the animals in a perfectly normal state. After some days they were less sensitive to mechanical and chemical stimuli than during the first day of captivity.

The greater part of the experiments was carried out with Isophyllia,[2] which proved to be a very useful object because its polyps are large. In the literature the statement is often found that remnants of food are hardly ever to be detected in the gastric cavity of coral-polyps (cf. Duerden, 1902; Pratt, 1906; Carpenter, 1910). These statements, however, in most cases are based upon the study of preserved material. Owing to the strong contraction produced by the preserving fluids, such polyps have discharged the contents of the gastric cavity.

In the polyps of freshly collected colonies one may often find a small amount of mucous matter protruding from the stomodaeum. In Isophyllia I found that these small masses of protruding material consisted for the greater part of matter of indeterminable nature (detritus), but also contained diatoms (both living and dead) and remnants of appendages of small crustaceans. Usually nematocysts also are to be found in this extruded matter. Besides other material, zooxanthellae were invariably found in these mucous masses. Some of these yellow cells were in various stages of disintegration, but others were quite normal.

The entoderm of the oral disk, of the tentacles, and of the edge-zone of Isophyllia is crowded with zooxanthellae, as already stated by Duerden (1902).[3] These zooxanthellae are spheroidal yellow algae with a diameter of $8\frac{1}{2}$ to $11\frac{1}{2}$ μ. They have a light yellow color, which seems to be distributed uniformly through the contents of the cell, but probably is located only in the small chromatophores which are found in great numbers in contact with the cell wall of the alga. In the central part of the alga one or two—sometimes more—small, strongly refringent bodies are to be found, which consist of an amyloid substance (as shown by the iodine reaction). In the living state the nucleus is not to be seen.

These zooxanthellae are always found in the digestive organs of the polyps of Isophyllia which have been freshly collected. It is well known that in actinians digestion takes place only in the mesenterial filaments (Metschnikoff, 1880; Mesnil, 1901; Jordan, 1907, to quote only a few authors), and it is also here that we have to trace the digestion of the food in the Madreporaria. The mesenterial filaments in Isophyllia consist of the somewhat thickened free edges of the septa,

which are bordered by a thick margin. The latter part is almost circular in cross-section and consists chiefly of nematocysts (cf. also Duerden, 1902). We find that the food is taken up and digested in the region next to that of the nematocysts. In mesenterial filaments from freshly collected polyps this region is crowded with zooxanthellae. When examined under a low magnification, therefore, a dark band is found adjacent to the marginal part containing the nematocysts. In the remaining part of the septum only sparsely distributed zooxanthellae are present in the entoderm cells. Some of the yellow cells in the mesenterial filaments still have the same form and contents as those found in the tissues of the oral part of the polyp. The condition of the contents of the greater number of the algae, however, differs from that of normal ones. A large proportion of them are irregularly discolored, and the most of these also show small corpuscles of dark brown color in contact with the wall of the cell. In some cases the zooxanthellae have lost the spheroidal form, being more or less irregularly distorted. There is very little doubt that these abnormal algae present different stages in the process of disintegration caused by the digestive action of the coral-polyps. This view is also supported by the fact that such zooxanthellae are always present in the remnants of food which are found protruding from the stomodaeum of freshly collected specimens.

Feeding experiments were carried out with a large number of polyps belonging to different colonies of *Isophyllia dipsacea*. The food, consisting of the meat of mussels, was readily taken, especially by specimens which had not yet been many days in the laboratory. Usually I mixed litmus with the food, so as to be able to trace the food in the internal organs after it had been swallowed. This mixture of mussel-meat and litmus was as readily ingested as pure mussel-meat.

The experiments were made in diffuse daylight in the laboratory. I found that Isophyllia could ingest food in three different ways. (1) Often, especially in fresh specimens, the food produced a sinking of the oral disk and a contraction of the sphincter muscle, causing the edge-zone to form a kind of roof over the oral disk and the tentacles in the manner described by Carpenter (1910). (2) In other specimens, also in colonies which had been recently collected, another method was seen: the food caused the mouths of the polyps to open, and then by ciliary action the food was slowly passed down through the stomodaeum into the gastric cavity, whereupon the mouth was again closed. In this case the oral disk and the edge-zone underwent

almost no change, although the tentacles increased slightly in size. In general the colonies, the polyps of which had fed in this way, filled their gastric cavity with more water, the tissues thereby projecting a little more above the skeleton. (3) The third manner of feeding observed in Isophyllia was the one which was usual for polyps of colonies which had been four or more days in the laboratory, though it also occurred sometimes in freshly collected specimens. When mussel-meat was placed on the oral disk of the polyps, mesenterial filaments protruded through the mouth and through different parts of the oral disk, and especially through the tissues of the edge-zone. When the mesenterial filaments came into contact with a piece of meat, the meat became more or less enveloped by these organs and partly ingested. This variability in the method of feeding observed in different polyps of Isophyllia was, perhaps, partly caused by the unnatural state of the animals due to the infrequency with which the sea-water was changed.

In a great many of the mesenterial filaments of polyps which had been fed with the mixture of mussel-meat and litmus red spots were found after some hours in the part next to the marginal zone of nematocysts, which proved that it was at this point that the meat had been ingested (the litmus accompanied the meat only as an indicator), and, furthermore, that the reaction in the food-vacuoles was acid. The same phenomenon has been found in actinians (Mesnil, 1901). In some of the mesenterial filaments no red vacuoles were present. In these large polyps of Isophyllia, therefore, only a part of the mesenterial filaments had ingested the food.

During several days the litmus remained in the zone of ingestion of the mesenterial filaments. After about three days the greater part of the litmus-colored vacuoles had assumed a blue color, showing that the reaction had changed from acid to alkaline. The final digestion and resorption of the food probably took place at this stage.

As I already have pointed out, in freshly collected, not artificially fed, polyps the mesenterial filaments are crowded, in the part next to the free margin, with zooxanthellae in various stages of disintegration. In the mesenterial filaments that had ingested a fairly large quantity of mussel-meat (as shown by the large number of litmus-colored vacuoles) only a very small number of zooxanthellae were to be seen a few days after the feeding. The few zooxanthellae still found in these organs never looked like those in the entoderm of the oral parts of the polyp; they were always in an advanced stage

of disintegration showing brown corpuscles and white spots. From these facts we may conclude that after abundant feeding on mussel-meat no more zooxanthellae had been ingested by these mesenterial filaments, and that the algae present in them before the feeding of the meat were, with a few exceptions, completely digested. This conclusion is further supported by the fact that in artificially fed polyps some of the mesenterial filaments do not ingest the colored mussel-meat. In these filaments, however, there is a zone full of zooxanthellae next to the marginal part.

Summarizing the above facts, we may describe the feeding of Isophyllia in the following way. Under normal circumstances the food of the polyp consists chiefly of the algae which are living in its entoderm cells. Besides yellow cells, small planktonic organisms are captured and digested, but food from this source seems under normal conditions to be too scanty to serve as exclusive nutriment for the polyps. When, however, the digestive organs (the mesenterial filaments) can obtain a sufficient quantity of food from other sources, they do not ingest any more zooxanthellae. The algae which were already present in the mesenterial filaments are gradually and completely digested, so that after abundant feeding on meat the digestive organs may become wholly devoid of zooxanthellae. Consequently the feeding of the polyps on zooxanthellae appears to be a condition due to the scarcity of food of extraneous origin. The symbiosis of coral-polyps and yellow cells represents, therefore, at least partly, a parasitic relationship between the two organisms, the polyps parasitizing on the zooxanthellae. Probably, on the other hand, the algae derive some profit from living in the entoderm, especially in that of the oral part of the polyps, the advantage consisting in their being exposed to the light and, perhaps, in their obtaining waste nitrogenous products from the polyp. At least, they thrive here quite well and increase rapidly in number by fission. The final result of this symbiosis is that the surplus of yellow cells is constantly eaten by the polyp.

These experiments have led to a result quite similar to that obtained by Van Trigt (1919) in fresh-water sponges. In the amoebocytes of these sponges Van Trigt found symbiotic algae (zoochlorellae) in various stages of being digested. In nature, however, the sponges digest only few symbiotic algae, their food supply of other origin being quite sufficient. When they were kept in aquaria the number of zoochlorellae which were digested by the amoebocytes considerably increased, owing to the lack of food from other sources. In

Isophyllia, on the contrary, under normal circumstances the extraneous food supply usually seems to be not very abundant, and therefore the zooxanthellae are largely eaten by the polyps. The algae are left unmolested only when enough food of other kinds is available.

I also made some feeding experiments on *Siderastraea radians*. A thick juice was obtained by pounding up the internal organs of mussels. This juice after being thoroughly mixed with litmus was poured over the surface of the colonies with a pipette. This colored food substance was readily ingested by the polyps. After a few hours a marked contrast between the artificially fed colonies and those which had not obtained mussel-juice was to be seen. In the former the polyps expanded so as to rise a little above the skeleton, with slightly extended tentacles, whereas in the unfed colonies the polyps remained in a contracted state.

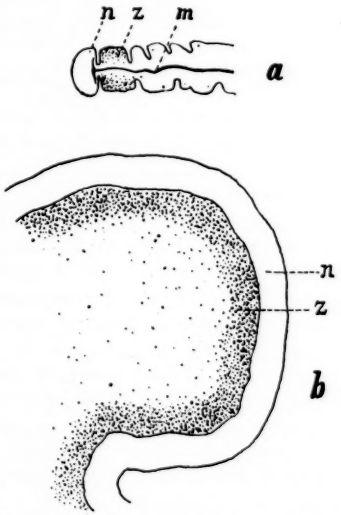
It was more difficult to experiment with this animal than with Isophyllia. Owing to the smallness of the polyps, the mesenterial filaments were far less easily obtained. Therefore the experiments on Siderastraea were much less complete than those on Isophyllia. On the whole, however, the results were quite the same. In freshly collected polyps of Siderastraea the mesenterial filaments showed a zone next to the marginal part crowded with partly digested zooxanthellae. After feeding mussel-juice and litmus abundantly, the litmus became visible as red spots in this zone, while the zooxanthellae gradually disappeared in the course of a few days. The symbiotic algae in the entoderm cells of the oral part of the polyps of Siderastraea have the same structure as those of Isophyllia.

Numerous zooxanthellae are also found in *Zoanthus sociatus*.[4] They occur in the entoderm of the oral zone, of the tentacles, and of the column. In size, color, and structure these algae agree completely with those of the two species of Bermudian Madreporaria in which I have studied the yellow cells, as recorded above. They contain usually one, often two, and only rarely more corpuscles consisting of amyloid substances. The mesenterial filaments of Zoanthus have a dark brown color, for they are completely filled with zooxanthellae. Many of these still have the same form and contents as those in the tissues of the oral part of the polyp. The yellow cells in the mesenterial filaments, however, are for the greater part more or less digested, their contents having assumed a different shape. In the interior of these zooxanthellae are found dark brown corpuscles and discolored spots, just as in the yellow cells in the mesenterial filaments of Isophyllia. The zone of the mesenterial filaments of Zoanthus in

which the digestion takes place is very broad; it lies next to the uncolored marginal part, which contains a large quantity of nematocysts.[5]

The feeding experiments on this species gave entirely negative results. The polyps refused to ingest mussel-meat or mussel-juice, and reacted only with strong contraction when these substances came in contact with either oral disk or tentacles. Meat of Chiton and of Fissurella was also invariably refused.

In the large sea-anemone *Condylactis passiflora* the entoderm of the oral disk and the tentacles is richly supplied with zooxanthellae. The brown color of the tentacles is entirely due to the presence of these algae. The yellow cells are quite similar to those of the other species dealt with in this paper. Usually they have the same size (average diameter 10 μ), though some smaller ones also occur among the others. The cells contain, one, two, or more amyloid corpuscles.[6]



Parts of mesenterial filaments of *Condylactis passiflora*, showing the distribution of the zooxanthellae in these organs. **a**, transverse section of a preserved, strongly contracted filament; **b**, part of a living mesenterial filament, in side view.

m, mesogloea; *n*, free edge with nematocysts; *z*, digestive part with zooxanthellae (indicated as black dots). × ca. 50.

The mesenterial filaments of Condylactis contain a large number of zooxanthellae in the region next to the colorless marginal part where nematocysts are found in great numbers (cf. the accompanying figure). In the remaining part of the mesentery only sparsely distributed zooxanthellae are present. The zone where great nunbers of zooxanthellae are found is doubtless the region where digestion chiefly takes place. Some of the yellow cells of this region, as well as those of the tentacles, are quite normal in appearance, but the larger part of them show various stages of disintegration, so that there results a discoloration of a portion of the cells and the formation in them of dark brown corpuscles. Consequently in Condylactis also the zooxanthellae seem to constitute an important part of the normal diet of the polyps.

As I collected these actinians on one of the last days of my stay in the Bermudas, I had no time to undertake feeding experiments with colored food. Had I been able to remain longer, the experiments with the large polyps of this species would doubtless have yielded good results, for the animals readily eat mussel-meat.

Summary.

The zooxanthellae of *Isophyllia dipsacea*, *Siderastraea radians*, *Zoanthus sociatus*, and *Condylactis passiflora* have approximately the same size and structure, and probably belong to one species of algae.

In the digestive organs (the mesenterial filaments) of freshly collected polyps of the four species mentioned large numbers of zooxanthellae are always found. They are in different stages of disintegration owing to their being in process of digestion by the polyps.

When the polyps of Isophyllia and Siderastraea are abundantly fed with mussel-meat, this is ingested by the mesenterial filaments. Under this condition the mesenterial filaments no longer ingest zooxanthellae. As a result the algae gradually disappear from the mesenterial filaments after the polyps are fed with meat. This suggests that the symbiotic algae are ingested by the polyps only when other food is scarce. In other Bermudian Anthozoa which live in symbiosis with zooxanthellae these algae also form a large part of the food supply of the polyps.

Consequently we may safely conclude that in general the symbiosis of Anthozoa with the algae known as "yellow cells" consists, in large part at least, of a parasitism of the polyps on the zooxanthellae.

Literature.

Boschma, H.
1924. On the Food of Madreporaria. Proc. Kon. Akad. Wetensch. Amsterdam, vol. **27.**

Carpenter, F. W.
1910. Feeding Reactions of the Rose Coral (Isophyllia). Proc. Amer. Acad. Arts and Sci., vol. **46.**

Duerden, J. E.
1902. West Indian Madreporarian Polyps. Mem. Nat. Acad. Sci., vol. **8.**

Fulton, Jr., J. F.
1921. Concerning the Vitality of Actinia bermudensis: a Study in Symbiosis. Jour. Exp. Zoöl., vol. **33.**

Gardiner, J. S.
1903. The Maldive and Laccadive Groups, with Notes on other Coral Formations in the Indian Ocean. Fauna and Geogr. of the Mald. and Lacc. Arch., vol. **1**, Part 4, p. 421–422.

Hickson, S. J.
1906. Coelenterata and Ctenophora. The Cambridge Natural History, vol. **1.**

Jordan, H.
1907. Die Verdauung bei den Aktinien. Arch. f. ges. Physiologie, Bd. **116.**

McMurrich, J. P.
1889[a]. The Actiniaria of the Bahama Islands, W. I. Jour. Morph., vol. **3.**
1889[b]. The Actinology of the Bermudas. *In* A. Heilprin, The Bermuda Islands. Philadelphia, p. 105–135, pls. 10–11.

Mesnil, F.
1901. Recherches sur la Digestion intracellulaire et les Diastases des Actinies. Ann. Inst. Pasteur, Tome **15.**

Metschnikoff, E.
1880. Über die intracellulare Verdauung bei Coelenteraten. Zool. Anz., Bd. **3.**

Pratt, E. M.
1906. The Digestive Organs of the Alcyonaria and their Relation to the Mesogloeal Ceil Plexus. Quart. Jour. Micr. Sci., vol. **49.**

Van Trigt, H.
1919. A Contribution to the Physiology of the Fresh-water Sponges (Spongillidae). Tijdschr. Nederl. Dierk. Ver., ser. 2, Deel **17.**

Verrill, A. E.
1900. Additions to the Anthozoa and Hydrozoa of the Bermudas. Trans. Conn. Acad. Arts and Sci., vol. **10.**
1901[a]. Variations and Nomenclature of Bermudian, West Indian, and Brazilian Reef Corals, with Notes on various Indopacific Corals. Trans. Conn. Acad. Arts and Sci., vol. **11.**
1901[b]. Comparisons of the Bermudian, West Indian, and Brazilian Coral Faunae. Trans. Conn. Acad. Arts and Sci., vol. **11.**

References.

[1] Also Hickson (1906) states that reef-corals perhaps derive in some cases a considerable part of their food from their symbiotic algae. In my former paper (Boschma, 1924) I have wrongly interpreted Hickson's views, inasmuch as I stated that this author denies the possibility that reef-corals feed exclusively on zooxanthellae. Hickson has only stated that he thinks it *improbable* that polyps with a complete set of organs for catching, swallowing, and digesting animal food never use them.

[2] Throughout this paper I have used the generic name Isophyllia for this coral, since it is by this name that it is generally known. Now, however, this genus is incorporated in the larger genus Mussa (cf. Verrill, 1901[b]). The specimens used in my experiments all belonged to the species *I. dipsacea*, which is to be distinguished from *I. fragilis* by its thicker septa and shallower calicles (Verrill, 1901[a]).

[3] The occurrence of a holophytic organism in certain species of Isophyllia is also recorded by Fulton (1921). According to this author the holophyte is probably allied to zooxanthella. A comparison of the symbiotic algae of Isophyllia with those of Zoanthus and Condylactis showed, however, that in all three genera these organisms are fairly alike in form, size, and structure.

[4] The form, color, and structure of my specimens agree completely with the description of *Zoanthus sociatus* by McMurrich (1889[a]). This species, however, is not mentioned by the same author (McMurrich, 1889[b]) in his report on the Bermudian actinians. Verrill (1900) records *Zoanthus sociatus* from the Bermudas. The number of tentacles in my specimens was 55–60, which is one of the specific features of *Z. sociatus* (cf. Verrill, 1900).

[5] Already in 1889 McMurrich (1889[b]) ascribed to this part of the mesenterial filaments a digestive function. The green corpuscles which he found in the mesenterial filaments were in all probability partly digested zooxanthellae. His studies were made on material preserved in alcohol. The yellow color of the zooxanthellae had been readily extracted by the alcohol, but the green color, which is less easily soluble therein, had remained.

[6] The zooxanthellae found in the species dealt with in this paper have the same size and color as those of the Madreporarian polyps of the East Indian region (cf. Boschma, 1924). The latter, however, generally contain one corpuscle of amyloid substance, and only very rarely two. In the Bermudian zooxanthellae often two or more of these corpuscles are present.